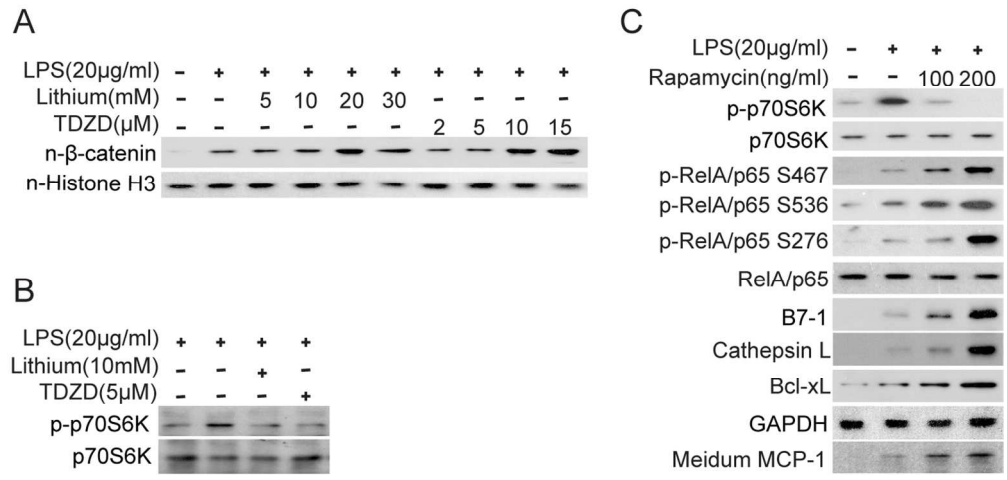
Supplementary Figure 1



Supplementary Figure 1. β-catenin and mTOR pathways are unlikely to contribute to GSK3β regulation of NFκB/RelA p65 in podocytes.
(A) Podocytes were pretreated with GSK3β inhibitors like lithium chloride or TDZD-8 at indicated doses for 20 minutes and then stimulated with LPS (20μg/ml) for 24 hours. Nuclear protein fractions were prepared from cells and subjected to immunoblot analysis for nuclear β-catenin (n-β-catenin) or for nuclear protein histone (n-Histone H3), which served as loading controls. The LPS induced nuclear translocation of β-catenin was augmented only by high doses of lithium or TDZD-8, whereas low doses of these GSK3β inhibitors, which were applied in the present study, barely affected the nuclear expression of β-catenin. (B) Podocytes were pretreated with GSK3β inhibitors like lithium chloride (10mM) or TDZD-8(5μM) for 20 minutes and then stimulated with LPS (20μg/ml) for 24 hours. Whole cell lysates were prepared and subjected to immunoblot analysis for indicated molecules. GSK3β inhibition by lithium or TDZD-8 resulted in an inhibition of the LPS activated TSC2/mTOR pathway, as indicated by the diminished phosphorylation of the mTOR substrate p70S6K. (C) Podocytes were pretreated with rapamycin, a standard specific inhibitor of the mTOR pathway, at indicated doses for 20 minutes and then stimulated with LPS (20μg/ml) for 24 hours. Immunoblot analysis for indicated molecules was carried out on cell lysates or conditioned media. Inhibition of the TSC2/mTOR pathway by rapamycin amplified the LPS induced NFκB activation and the expression of all NFκB target molecules in a dose dependent fashion.
126x73mm (300 x 300 DPI)